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RYR1-related rhabdomyolysis: a common but probably underdiagnosed manifestation of skeletal muscle ryanodine receptor dysfunction

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Abstract

Mutations in the skeletal muscle ryanodine receptor (*RYR1)* gene are associated with a wide spectrum of inherited myopathies presenting throughout life. Malignant Hyperthermia Susceptibility (MHS)-related *RYR1* mutations have emerged as a common cause of exertional rhabdomyolysis, accounting for up to 30% of rhabdomyolysis episodes in otherwise healthy individuals. Common triggers are exercise and heat and, less frequently, viral infections, alcohol and drugs. Most subjects are normally strong and have no personal or family history of malignant hyperthermia. Heat intolerance and cold-induced muscle stiffness may be a feature. Recognition of this (probably not uncommon) rhabdomyolysis cause is vital for effective counselling, to identify potentially malignant hyperthermia-susceptible individuals and to adapt training regimes. Studies in various animal models provide insights regarding possible pathophysiological mechanisms and offer therapeutic perspectives.

Keywords

Skeletal muscle ryanodine receptor (RYR1) gene; ryanodine receptor (RyR1); exertional rhabdomyolysis; exercise; rhabdomyolysis; genetic; myopathy

1. Introduction

Mutations in the skeletal muscle ryanodine receptor (*RYR1*) gene are associated with a wide spectrum of inherited myopathies presenting throughout life [1], including dominantly inherited Central Core Disease (CCD) [2], and subgroups of recessively inherited Multi-minicore Disease (MmD) [3], Centronuclear Myopathy (CNM) [4] and Congenital Fibre Type Disproportion (CFTD) [5]. The dominantly inherited Malignant Hyperthermia Susceptibility (MHS) trait, a pharmacogenetic predisposition to severe and potentially life-threatening reactions in response to volatile anaesthetics and succinylcholine (for review, [6]), is an allelic condition; some patients with CCD may also be MH-susceptible, and MH-susceptible individuals may feature cores on muscle biopsy even without being weak.

The majority of MH-susceptible individuals are clinically asymptomatic unless exposed to triggering agents, however, several permanent or induced muscular manifestations appear to be specifically associated with MHS-related *RYR1* mutations: King-Denborough syndrome (KDS) is an early-onset myopathy with short stature, scoliosis, dysmorphic features and a predisposition to MH reactions [7]. More recently, (MHS-related) *RYR1* mutations have also emerged as a common cause of exertional rhabdomyolysis (ERM), accounting for up to 30% of rhabdomyolysis (RM) episodes in otherwise healthy individuals [8]. This review will focus on the features of *RYR1*-related (E)RM by discussing the key features from cases reported to date, summarizing the proposed pathophysiological mechanism(s) extrapolated from observations in relevant animal models, and suggesting how this probably not uncommon manifestation of *RYR1* mutations can be recognized and how it should be managed.

2. (Exertional) rhabdomyolysis

RM (from the Greek, literally the “dissolution of striated muscle”) is the general term for muscle breakdown associated with a wide variety of external triggers, including strenuous exercise beyond the limit of fatigue, inadequate hydration, high ambient temperatures, use of supplements (ephedra,

creatine, and herbal weight loss supplements) or certain medications (statins, selective serotonin reuptake inhibitors), illicit drug or alcohol abuse, muscle trauma, or recent viral illnesses. Although there is no universally accepted definition, RM is often defined as a clinical syndrome associated with severe muscle pain, sudden elevation and subsequent fall of serum creatine phosphokinase (CPK) levels with or without myoglobinuria. There is no consensus concerning the exact level of CPK rise required to fulfil the definition of RM, with suggested CPK levels ranging from > 5, > 10, > 20 to even > 50 times the upper limit of normal (ULN) [9]. ERM is defined as RM preceded by triggering strenuous exercise (often under environmental conditions at the extremes of the temperature scale). RM results in the entry of skeletal muscle contents, in particular CPK and myoglobin, into the systemic circulation. The course is mostly characterized by myalgia with mild to moderate CPK increases, mild asymptomatic pigmenturia, and will often not even come to medical attention. However, in a minority of patients the clinical course is severe, resulting in profound hyperCKaemia, acute renal failure, compartment syndrome, disseminated intravascular coagulation, cardiac arrhythmias secondary to electrolyte imbalances, and even cardiac arrest if left untreated. The degree of metabolic disturbances and the severity of RM-related clinical features depends on the amount of muscle damaged and various endogenous and exogenous factors (such as hypovolemia, hypotension, body temperature, sepsis, intake of toxic drugs, general health, hormone environment and genetic susceptibility). Irrespective of its cause, the pathophysiological events in RM follow a common pathway, summarized in Box 1. The annual RM prevalence has been reported as 26,000 cases in the United States, with 47% meeting the diagnostic criteria of ERM [10].

Box 1: Pathophysiology of exertional rhabdomyolysis (ERM)

Normally, ion pumps and channels in the sarcolemma maintain a low intracellular Na^+ and Ca^{2+} and a high intracellular K^+ concentration. Unaccustomed (eccentric) exercise may cause direct injury to the sarcolemma and/or lead to failure of energy production with subsequent pump dysfunction of Na^+/K^+ATPase and Ca^{2+}ATPase. This leads to increased cellular permeability to sodium ions and, consequently, increased intracellular calcium concentrations, with subsequent muscle contraction increasing the energy deficit. This also enhances the activation of calcium-dependent proteases and phospholipases, which contributes to destruction of myofibrillar, cytoskeletal and membrane proteins. Subsequently, large quantities of intracellular electrolytes, metabolites as well as

intracellular proteins (aldolase, myoglobin, CK, lactate dehydrogenase, aspartate transaminase) leak into the circulation. The resulting free calcium will add to the contraction of the already over-activated myocytes.

Electrolyte disturbances normally occurring during exercise, e.g. hypokalemia (through perspiration) and hyponatremia (due to polydipsia) reinforce this process. Potassium release from muscle cells during exercise normally mediates vasodilation and an appropriately increased muscle blood supply. Hence, hypokalemia may promote the development of RM by decreasing blood flow to muscles in response to exertion, thus limiting availability of energy sources. Next, potassium leak from myocytes into the bloodstream causes hyperkalemia, which can result in fatal cardiac arrhythmias. Hyponatremia contributes to Na^{+}/Ca^{2+}-ATPase dysfunction, which leads to the activation of proteases and lipases that are responsible for further cell lysis. Furthermore, hyponatremia causes failure of cell volume regulation. Combined with other cellular responses to exercise such as increased oxidative stress and release of pro-inflammatory cytokines, this will ultimately result in cell death and intracellular components spilling into the surrounding tissue. This is perpetuated by extreme ambient temperatures. The prolonged exposure to very low temperature causes vasoconstriction and subsequent cell damage. On the other hand, hyperthermia is considered as a hypermetabolic condition that triggers RM when the energy supplies are not adequate in relation to cellular requirements.

RM often represents a “physiological” response to extreme external triggers, such as fasting, viral infections, toxins or medication, strenuous physical exercise, exercise under extreme circumstances, or a combination of these factors [11]. In contrast, ERM might also be the first manifestation of a genetic muscle disorder, reflective of the potential of such conditions to lower the trigger threshold for developing muscle breakdown. A genetic cause should always be considered in case of recurrent, familial or paediatric episodes of ERM presenting very early in life; if such episodes are associated with other (exercise-induced) muscle symptoms such as cramps, myalgia, exercise intolerance, hyperCKemia in the personal or family history, or if the severity of RM exceeds the expected response to the exercise. The recognition of a patient with an underlying genetic disorder is essential for the acute management but also (and most importantly) for counselling afterwards. Several genetic disorders increase the risk of developing RM. These include metabolic myopathies, disorders of calcium homeostasis, various muscular dystrophies (particular limb girdle muscular dystrophies such as LGMD2I and milder dystrophinopathies), and the sickle cell trait. We refer to recent detailed reviews for an overview of genetic disorders to be considered where the nature of the RM episodes raises the suspicion of such a condition [12-14].

3. *RYR1*-related (exertional) rhabdomyolysis ((E)RM)

A link between ERM and MHS had been suggested since the original description of the latter but until recently had only been documented in case reports and small series. Such a link is plausible, considering 1) an uncontrolled rise in intracellular skeletal muscle calcium as the most important shared pathomechanism [15]; and 2) the common occurrence of MH and ERM in a number of porcine [16], equine [17] and murine [18, 19] *RYR1* mutants.

Wappler et al. were the first to perform a contracture test (the standard diagnostic test for MH since the mid 1970-s, consisting of the in vitro measurement of contracture response of biopsied muscle to graded concentrations of caffeine and halothane; it is referred to as the caffeine/halothane contracture test (CHCT) in North America and the in vitro contracture test (IVCT) in Europe) in 12 unrelated patients with ERM in 2001, ten of which had positive results; one patient had a negative test result, and one patient showed equivocal responses [20]. Three of these patients had RYR1 mutations but only hot-spots sequencing had been performed at the time [20]. Based on their findings, the authors recommend performing genetic testing for MH-associated *RYR1* mutations, muscle biopsies for histological examination and IVCT in patients presenting with ERM. In 2009, Sambuughin sequenced the *RYR1* gene in six unrelated African-American men with unexplained ERM, who were subsequently diagnosed as MHS on CHCT. Three novel mutations and two variants previously reported in Caucasian MH-Susceptible subjects were found in five patients studied, further emphasizing the importance of performing CHCT and *RYR1* mutation screening in patients with unexplained ERM [21]. In our seminal study systematically investigating a possible link between ERM and *RYR1* mutations in 2013, *RYR1* was sequenced in 39 unrelated families with RM, in whom common metabolic and mitochondrial causes had been excluded [8]. In this series we identified nine heterozygous *RYR1* mutations/variants in 14 families, five of them (K1393R; G434R; T4288_A4290dup; A4295V; and R4737Q) previously associated with MH. Index cases presented from 3 to 45 years with RM with or without exertional myalgia (n=12) or isolated exertional myalgia (n=2). Familial *RYR1* mutations were confirmed in relatives with similar, exertional myalgia without RM, or

no symptoms. Common triggers were exercise and heat and, less frequently, viral infections, alcohol and drugs. Most subjects were normally strong and had no personal MH history. Some reported heat intolerance and cold-induced muscle stiffness. Muscle biopsies showed mainly subtle changes. These findings suggest that *RYR1* mutations account for a substantial proportion of patients presenting with unexplained RM and/or exertional myalgia, in particular in association with the reported triggers. Associated features may be subtle, including prior exertional myalgia, mild hyperCKemia, mild axial weakness or subtle (unilateral) ptosis, and some degree of unevenness of oxidative staining on muscle biopsy. Family counselling is vital to identify potentially MH-susceptible relatives [1]

We performed a systematic literature review (including the patients in the studies mentioned above), based on a Pubmed search with "*RYR1*" and "rhabdomyolysis". This resulted in identification of several case report and small series [1, 8, 21-26], and two review article [27, 28] Subsequently, we also included cases from references of these articles [20, 26, 29-34] and added our recent letter by Snoeck et al. [35]. Finally, we added two patients from our paper on the spectrum of *RYR1* related myopathies by Snoeck et al. who were not included in any other publication (patients 14 and 52[1]). We excluded the patients who suffered only from exertional myalgia without clinical or biochemical RM (Family 11 and 14 in Dlamini et al.[8]). We identified 37 patients with non-anaesthetic RM due to *RYR1* mutation(s) (age range 1 – 64 years; 31 males and 6 females). The most important features from these cases are summarized in Table 1, and an illustrative case reported recently is presented in more detail in Box 2 [35].

Box 2: *RYR1*-related exertional rhabdomyolysis (ERM) in a 16-year old patient – case study

This patient is a currently 21-year-old semi-professional male cyclist who has experienced three episodes of RM after cycling [35]. The first episode occurred at the age of 14, when he developed profound myalgia in his legs four days after a race, resulting in impaired walking. He recovered within one week and continued with cycling training without seeking medical advice. When he had the same symptoms two years later, his CPK was found to be elevated at 29,914 IU/l and he was admitted to the ICU for rehydration. He was referred at the age of 17. He reported that the race where the ERM episode occurred had taken place in very cold weather, but that he had competed under similar conditions before. Most likely, the combination of several triggers (use of NSAIDs because of mild flu-like symptoms before the race; very cold temperature; strenuous training preceding the race; and use of a caffeine gel as an energy booster) had caused the ERM episode

after this particular race. Physical examination revealed an athletic build but mild weakness of the rhomboid muscle (MRC 4)(Figure 3). He also had mild bilateral ptosis. The muscle biopsy (including EM) showed an increase of internal nuclei but no cores. *RYR1* sequencing revealed compound heterozygous *RYR1* mutations, I2321V (maternal allel) and V4849I (paternal allel) previously associated with MH. The IVCT was positive, confirming MHS. After he was diagnosed with *RYR1*-related ERM, he adapted his training regime and refrained from use of caffeine and NSAIDs. Nevertheless, at the age of 18 he experienced a third ERM episode with severe myalgia and significant hyperCKaemia (10,820 IU/l), and he subsequently gave up semi-professional cycling.
His father carried the G14545A mutation and was also very athletic but never experienced similar problems. However, his paternal uncle (G14545A carrier) had visited our centre at the age of 37 because of hyperCKemia, fatigue and muscle cramps. He was also very athletic (regular cycling, rugby) and had experienced exertional myalgia and muscle stiffness since the age of 35. CPK levels were around 800 IU/l. The muscle biopsy had shown mild non-specfic myopathic features (increase in fibre size variability and internal nuclei but no cores). Physical examination at the age of 49 showed an athletic build, no muscle weakness but a mild ptosis (Figure 3). After genetic testing confirmed the G14545A mutation, he was counselled for the MHS risk and referred to the rehabilitation specialist for training advice. All first degree relatives were screened for the G14545 mutation because of the MHS risk.

The review of these cases revealed very important insights into the characteristic features of *RYR1*-related RM. First, RM manifested ***at all ages***, with a fatal non-anaesthetic MH reactions in six children. There was a ***male predominance*** (84%). In many cases, RM was not provoked by the first exposition to a specific trigger and often a ***combination of variable triggers*** (including recreational and medical drugs) was present, both in children and adults, e.g. physical exercise and hot ambient temperatures, or viral infection or exercise and medication. Gener et al. reported the case of a child who had suffered a sevoflurane-triggered MH episode treated with dantrolene during an orchidopexy procedure (at the age of 3 years 5 months), a common operation during which MH episodes have been observed [32]. He died at the age of 5 and a half years when he developed muscle rigidity and became hyperthermic and asystolic after receiving ondansetron because of vomiting and abdominal pain [32]. A recent case published by Russell et al. illustrates a similar point: a 20-year-old woman who developed RM, disseminated intravascular coagulopathy and multi-organ failure induced by MDMA. Following initial improvement, she developed delayed RM due to haloperidol-induced neuroleptic malignant syndrome (NMS), which was treated with dantrolene. Subsequent genetic testing revealed a novel potentially pathogenic variant in *RYR1* (G2545A). The

case reported by Lavezzi showed how succinylcholine augmented the hypermetabolic state resulting from heat stroke, leading to subsequent death [33]. Furthermore, Forrest reported an MH event preceded by unaccustomed exercise, suggesting that MH may in fact also be a multifactorial event and providing a potential explanation for its highly variable penetrance [36].

These observations support the ***suspected relationship between exertional heat illnesses, ERM, and MHS*** (Box 3) [15, 25], with a ***fatal course especially in children*** illustrated in several reports. Nishio et al. presented a young patient (2 years 9 months) that died of heat stroke after being left in a car in high environmental temperature. Postmortem mutation analysis revealed that the child carried compound heterozygous *RYR1* mutations L4320_R4322dup and R4645Q. Since each mutation had previously been documented to cause MHS on its own, a non-anaesthetic MH-like response prompted by high environmental high temperature might have occurred in this child essentially carrying a "double-dose" of MHS-related *RYR1* mutations [30]. Groom et al. reported a de novo *RYR1* variant (R3983C) in two unrelated children who experienced fatal, non-anesthetic MH episodes associated with febrile illness and heat stress. One of those children had numerous episodes of leg cramping, tachycardia, tachypnea and increased creatinine kinase years before the fatal episode [29]. Tobin reported a 13-year old patient who died of a stress-induced hyperpyrexic death without anaesthetic exposure after a football game with an ambient temperature of 26°C. One and a half year prior, he had developed an MH response to general anaesthesia, for which he was successfully treated with dantrolene. Post mortem *RYR1* sequencing revealed a *RYR1* mutation (R163C) in the patient and, on further testing, in his father [31]. The children reported by Gener and Lavezzi also had a fatal outcome of their heat illness.[32, 33] This clinical overlap has implications for some MH-susceptible patients and their capacity to exercise, as well as for clinicians treating and anesthetizing patients with histories of unexplained exertional heat and exercise illnesses [25].

Box 3: Exertional heat illness, exertional rhabdomyolysis (ERM), and malignant hyperthermia susceptibility (MHS) [15, 25, 37]
Exertional heat illness, ERM, and MH have similar pathophysiology: all three are hypermetabolic

states that include high demand for adenosine triphosphate, accelerated oxidative, chemical, and mechanical stress of muscle, and uncontrolled increase in intracellular calcium.

Heat related illnesses:

1. **Heat stroke**: a severe condition of hyperthermia (body temperature > 40.6 °C (105.1 °F)) due to environmental heat exposure with lack of thermoregulation. The term "stroke" in "heat stroke" refers to the altered mental status that accompanies this hypermetabolic condition. Prevention consists of drinking plenty of cool liquids and avoiding excessive heat and humidity, especially in unventilated spaces. Treatment requires rapid physical cooling of the body.
2. **Heat exhaustion:** a condition of heavy sweating and tachycardia, resulting in dehydration, salt-depletion, and hypovolaemia. Causes include exposure to high temperatures, particularly when combined with high humidity, and strenuous physical activity. Without prompt treatment, heat exhaustion can lead to heatstroke.
3. **Heat syncope:** fainting due to peripheral vasodilatation secondary to high ambient temperature. It occurs with or without mental confusion.
4. **Heat cramps:** Painful, brief muscle cramps that occur during exercise or work in a hot environment or begin a few hours later. They are likely to be related to electrolyte problems due to extensive sweating and rehydration with large amounts of water or other fluids that lack salt.

Exertional rhabdomyolysis (ERM): the breakdown of muscle from extreme physical exertion. It is a common complication of exertional heat illness, but may also occur in the absence of features of exertional heat illness. There is a clear association with *RYR1* variants, including variants associated with MH susceptibility. Risk factors: strenuous exercise under high temperatures and humidity; poor hydration; inadequate recovery between exercise, inadequate fitness levels.

Malignant hyperthermia (MH): a rare life-threatening condition that is characterized by rapid rise of end-tidal CO_2 and body temperature occuring in susceptible individuals who receive volatile anesthetic agents and/or succinylcholine. In susceptible individuals, these drugs can induce a drastic and uncontrolled increase in oxidative metabolism as a result of a persistent intracellular Ca^{2+}, which devastate the capacity to supply oxygen, remove carbon dioxide, and regulate body temperature, eventually leading to circulatory collapse and death if not immediately treated.

These latter cases also point to the ***muscular symptoms that were present prior to the RM episode*** in many of the index cases**.** More than half of the patients we identified through our literature search reported prior exertional myalgia (Table 1). Two of the three families reported by Davis et al. also reported intermittent muscle pain and cramps, often precipitated by exercise. These episodes lasted for two weeks and were so severe that walking was impaired [26]. Finally, muscle biopsy showed signs of a congenital myopathy in only one patient and one carrier father; all other biopsies were normal or showed only aspecific changes.

Several reports show that ***muscular symptoms might also occur in other relatives compatible with an autosomal dominant inheritance pattern***. For example, Potts et al reported a 30-year-old man with persistent hyperCKemia, severe debilitating muscle cramps, and unexplained RM. The CHCT was positive, and subsequent *RYR1* sequencing revealed a mutation functionally causative for MH. His identical twin brother, who was suffering from similar complaints, was found to share the same mutation. It is important that such features are specifically asked for in the family history, as patients and their relatives may not necessarily consider those symptoms linked. The dominant inheritance differentiates this cause from metabolic causes of RM (in particular disorders of glycogen metabolism, disorders of long-chain fatty acid metabolism and mitochondrial myopathies; for review: [12-14]). Another distinguishing feature from the metabolic myopathies is that ***fasting was not a trigger*** in any of the *RYR1*-related cases. For example, a previously reported 44-year-old patient with recurrent, severe episodes of fever-induced RM (521,500 IU/L) had been exposed to several prolonged periods of religiously motivated fasting without experiencing any symptoms [23].

The case reported by Capacchione et al. illustrates that ***symptoms increase in the course of several days after the strenuous exercise*** [25]. This was also reported by the young cyclist in Box 2: in both episodes, the myalgia manifested three to four days after the race. This timeline is similar to the development of myalgia after unaccustomed exercise in healthy individuals, and parallels the CPK increase (Figure 2). Heat-induced RM generally develops more rapidly, which likely results from acid-base and plasma composition changes. The initial lactic acidosis and respiratory alkalosis evolves into a predominantly metabolic acidosis, reflecting tissue damage (release of sulphate, phosphate and organic acids).

Finally, individuals with *RYR1*-related RM are ***often particularly muscular and athletic***, making life style advice after a RM event more challenging. One of the patients reported by Dlamini et al. (*patient 7.II.2*;also *patient 23* in Snoeck et al.) had to change her plans to become a sports coach after the second ERM episode with CPK levels up to 378,900 IU/l [1, 8]. Similarly, the patient illustrated

above (Box 1) stopped cycling semi-professionally only after another episode of RM requiring hospital admission occurred a year after he was diagnosed with *RYR1*-related RM.

As a complicating factor, ***counselling might be challenging in cases with more than one RYR1 mutation***, especially where the pathogenicity of individual mutations is not defined yet. In the cohort we identified, nine patients had more than one mutation (Table 1). Another complicating factor in counselling is the often highly ***variable penetrance of the familial RYR1 mutation.*** The father of the patient in box 2 also carried the *RYR1* mutation and was very active in sports, but never experienced any RM episodes.[35] Even more remarkable is the carrier brother of patient 7.II.2 reported in Dlamini et al. (Figure 1 in that article), who is very active in weightlifting competition but never had any RM episodes [8].

A particular complex issue is the ***variable relationship between RYR1-related RM and the results of CHCT or IVCT testing.*** The mother of the patient reported by Russell [24] and two patients reported by Snoeck et al. (*patient 29* and *52*)[1], all with one or more episodes of RM, had negative results on the CHCT or IVCT, proving a lack of complete overlap between ERM and MHS. This test, which remains the only validated diagnostic test for phenotypical MHS, has several limitations. It was validated by comparing muscle biopsy tissue from patients with documented histories of anesthesia-related MH to muscle biopsy tissue from control patients resulting in a specificity of 93,6% and a sensitivity of 99% [38]. As a result, using the CHCT or IVCT to diagnose people as MHS who present with (E)RM, given that these tests were not developed or validated to evaluate these patients, might give false-positive results. In the three patients with negative tests, pathogenicity was assumed based on the presence of biallellic mutations (*patient 52*), the absence of the mutation in normal population, and/or the results of *in silico* analysis. Functional testing of the mutant RyR1 channels in a suitable expression system will be required to confirm the *pathogenicity* of mutations where CHCT or IVCT testing is equivocal or negative.

The case reported by Lavezzi et al illustrates the ***importance of recognizing RYR1-related heat illness****:* this healthy 6-year-old boy developed lower extremity rigidity, trismus, and fever after

playing in an inflatable swimming pool. On arrival in the emergency department, he appeared to be seizing. An endotracheal tube was urgently placed using succinylcholine to facilitate muscle relaxation, he went into cardiac arrest and resuscitation attempts were unsuccessful. Post mortem genetic analysis showed a novel *RYR1* variant, also confirmed in his father who had a positive CHCT response and central cores on the muscle biopsy [33]. This case, and that of Capacchione [25] exemplify that (E)RM is a presentation of *RYR1*-related myopathies ***most anaesthesiologists are not familiar*** with [25, 33]. Furthermore, most general practitioners, emergency department physicians, hospital doctors, and intensivists do not associate (E)RM with MH; thus, consideration of *RYR1* mutations as a cause may be easily forgotten. Although the incidence of fulminant MH during anaesthesia is relatively rare (with estimates between 1 in 4200 to 250,000), the incidence of *RYR1* mutations with MHS in the general population might be up to 1 in 2000 [5]. This implies that there is a cohort of possible MH-Susceptible individuals who do never develop MH during general anaesthesia but may well present with ERM at one point in life. In support of this point, exome sequencing performed on 870 volunteers without medical or family histories for MHS identified three *RYR1* variants predicted to predispose to MH reactions.[39]

Finally, the case reported by Potts et al illustrate ***the continuous use of dantrolene for treatment of cramps and prevention of RM*****:** these 30-year-old identical twin brothers with persistent hyperCKemia, severe debilitating muscle cramps, and unexplained RM both required oral dantrolene therapy to control their symptoms [34].

Pathophysiology. The precise pathophysiological mechanism(s) underlying human *RYR1*-related (E)RM are still unknown but investigations in relevant animal models may provide useful information: studies in the Y522S RyR1 KI mouse (a murine model of human MH) suffering RM and death in response to elevated environmental temperatures suggest a complex interplay and a vicious cycle involving the primary RyR1 defect and secondary metabolic RyR1 modifications: The murine Y522S RyR1 was shown to cause a Ca^{2+} leak, which drives increased generation of reactive nitrogen species;

the resulting S-nitrosylation of the mutant RyR1 receptor in turn further increases its temperature sensitivity, lowering the threshold for producing muscle contractures upon exposure to elevated temperatures. Many mitochondria in the muscle of heterozygous Y522S mice are swollen and misshapen, and the mutants display force reduction with aging. Assuming that mitochondria as a store of Ca2+ play a buffering role in controlling cytosolic Ca2+ metabolism under normal conditions, the authors propose a destructive feed-forward cyclic mechanism that increases the temperature sensitivity of RyR1 activation and underlies heat stroke and sudden death. They propose that this cycle eventually produces a myopathy with damaged mitochondria due to defects of the mitochondrial respiratory chain (Figure 4)[19]. The overall increase in RyR1 sensitivity to activation by stimuli that promote Ca^{2+} release render the muscle more susceptible to rhabdomyolysis to the presence of any trigger, among which is (eccentric) strenuous exercise.[18]

4. Management

Acute management. The first step when encountering a patient with a (E)RMis to determine whether the RM is clinically significant, requiring hospital admission and intravenous fluid administration. Although randomized controlled trials offering concrete management guidelines for these aspects of RM management do not exist, the study by Kenney at al. reporting a large cohort of representative cases with physiological ERM offers some practical guidelines at least for the initial steps (summarized in Box 4 and [9]).

Box 4: Features requiring hospital admission and intravenous fluid administration	
Clinical features	Myalgia with: • muscle weakness • swelling • altered consciousness
Exercise	Accustomed / unaccustomed
History	In patient or relatives: • prior episodes of ER • other indicators of NMD • renal, cardiac or co-morbidity
Vital signs	Abnormal

	Body temperature > 40°C (heat stroke)
CPK (U/l)	≥ 10,000
Myoglobinuria/myoglobinemia	Present
Acute renal failure	Absent or present
Electrolyte abnormalities	Absent or present
Acid base status	Abnormal
Other factors possibly provoking RM	Absent or present

The goals of both inpatient and/or outpatient treatment are to avoid renal injury, prevent further muscle injury, and to limit complications resulting from electrolyte disturbances; this is summarized in Box 5. Most importantly, in *RYR1*-related ERM cases, the specific RyR1 antagonist dantrolene can be administered to limit further muscle breakdown and CPK increases, and more severe, potentially life-threatening downstream medical complications. In addition, allopurinol has recently been suggested as a treatment for exercise-induced muscle damage [40]. Allopurinol is a purine hypoxanthine-based structural analogue and a well-known inhibitor of xanthine oxidase, an enzyme which generates free radicals during oxidative stress induced by intensive muscular activity. Its administration may hence be regarded as promising, safe, and an economic strategy to decrease transient skeletal muscle damage and limit RM.

Box 5: Management of exertional rhabdomyolysis (ERM) [41, 42]

Out-patient follow-up of patients with physiological exertional rhabdomyolysis (ERM)

Rest for 72 h and encourage oral hydration
Sleep 8 h consecutively, nightly
Prevent heat exposure: Remain in thermally controlled environment if ERM occurred in association with heat injury.
Refer to ER in case temperature exceeds 40 °C
Follow up in 72 h for repeat CPK and blood urea:

- CPK < 5 x ULN and normal blood urea: no further studies
- Return every 72 h and repeat until CPK < 5 x ULN and normal blood urea
- CPK ≥ 5 x ULN or abnormal blood urea for > 2 weeks: refer for expert consultation
- CPK ≥ 50 x ULN (10,000 U/l): refer to ER again

Refrain from sports
Elimination of medications, drugs and toxins that are considered to cause RM

Clinical management of patients with clinically significant RM

Administration of oral or iv dantrolene (dose dependent on severity of RM and hypermetabolic state)
Hyperhydration: Intravenous fluids should be initiated as soon as possible, preferably within the

first 6 hours after muscle injury, at a rate that maintains a urine output in adults of ≥ 300 mL/h for at least the first 24 hours
Admittance to ICU
P**eritoneal dialysis or hemodialysis** in patients with little or no urine output despite hyperhydration, profound acidosis, or severe hyperkalemia
Mannitol should be administered only to maintain urine output of 300 mL/h or more despite adequate fluid administration
Sodium bicarbonate should be administered only if necessary to correct systemic acidosis
Elimination of medications, drugs and toxins that are considered to cause RM

Low dose oral dantrolene could also be considered for the treatment of chronic muscle pain and prevention of recurrent episodes in individuals with MHS or *RYR1*-related RM without significantly affecting performance [44].

Restarting sports. Patients with *RYR1*-related ERM will have a higher recurrence risk and will have to be specifically advised with regards to subsequent sporting activities. If such patients choose to continue with exercise, they should be advised to try to avoid exercising at times of extreme environmental temperatures, during intercurrent (febrile) illness and/or when medical or recreational drugs are being taken. O'Connor suggests three phases of return to physical exercise for athletes who are not at risk: *Phase I*: onset to normalisation of CPK to < five times upper limit of normal; *Phase II*: start of light activities; *Phase III:* Gradually return to regular sporting activities and physical training (Guideline evolved from the Rhabdomyolysis working group at the Consortium for Health and Military Performance (CHAMP): Box 6) [43]. Patients with *RYR1*-related RM may also be at risk of MHS with important consequences for affected individuals and their relatives carrying the same *RYR1* variants.

Box 6: How to restart sports? [43]

First four weeks (Phase I)

- Begin light but no strenuous physical activities
- Follow up with physician in 1 wk
- In case of clinical symptoms (muscle weakness, swelling or myalgia) return to physician
- Remain in start schedule and return at 1-wk intervals

Advice to restart sport at least four weeks after the event and only if the patient is asymptomatic (Phase II -III:

- Only if no clinical symptom return (muscle weakness, swelling or myalgia) gradually increase the intensity and duration
- Avoid unaccustomed exercise, especially eccentric training
- Follow up with care provider as needed

Additional advices:

- Very gradual return to a less intense training schedule
- Search help of a physical therapist / sports medicine specialist / rehabilitation specialist with expertise of training in inherited myopathies
- Strictly avoid the combination of strenuous exercise with other risk factors for RM (drugs, medication, supplements, viral infection)
- Strictly avoid unaccustomed, strenuous exercise, especially eccentric training
- Prevent dehydration during exercise
- Prevent the combination of extreme heat and cold exposure and exercise: limit training intensity in hot and humid environment, and in very cold environments
- Report to physician in case of exertional myalgia and perform CPK check-up
- Do not use caffeine or other supplements
- Limit alcohol consumption in periods of intense sport activities
- Wear a SOS necklace

5. Concluding remarks

This review has focused on the specific features of *RYR1*-related (E)RM, a probably not uncommon but underdiagnosed form of RM. Various triggers, alone or in combination, can evoke *RYR1*-related RM, including physical exercise, especially if unaccustomed and/or performed under extreme environmental temperatures, viral infection, and medical and recreational drugs. These are in fact the same triggers known to evoke RM in general, but in the context *RYR1* mutations, the trigger threshold is lower, or RM events are more severe and/or recurrent. In contrast to some of the metabolic causes, inheritance is mainly dominant, myalgia generally does not occur during but following often considerable intervals (up to 72 hours) after exercise, and fasting has not been reported as a triggering factor. Patients may often be particular muscular and athletic, making lifestyle advice difficult. Individuals with *RYR1*-related RM may also be MH-susceptible, but the relationship with CHCT or IVCT results is not consistent, suggesting an area for further research. Recognition of *RYR1* mutations as a cause of rhabdomyolysis is vital for effective (family) counselling, to identify potentially MH-susceptible individuals and to adapt training regimes. Studies in various

animal models may provide insights into pathophysiological mechanism(s) of potential relevance also for humans, and offer therapeutic perspectives.

Figures

Figure 1: Spectrum of inherited myopathies and muscular conditions due to *RYR1* mutations in Dutch cohort (Reprinted with permission of the publisher (N=77)[1].

Figure 2: Temporal course of myoglobin and CPK levels during a typical rhabdomyolysis (RM) episode. Myoglobin is the first enzyme that increases, but returns to normal levels within the first 24 hours after onset of symptoms. CPK increases a few hours later, reaches its peak value within the first 24 hours, and remains at these levels for around 3 days. Even though the presence of myoglobin in serum is the key feature of RM, CPK is considered to be a more useful marker for the diagnosis and assessment of the severity of muscle injury due to its delayed plasma clearance and the wider availability for diagnostic testing.

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Figure 3: A 17-year old semi-professional cyclist with a history of recurrent *RYR1*-related ERM episodes. Note mild axial weakness. A paternal uncle carrying the same mutation had presented with exertional myalgia earlier in life, and was found to have an athletic build and mild unilateral ptosis on examination.

Figure 4: Possible pathophysiology of *RYR1*-related rhabdomyolysis, based on the model of exertional/environmental Heat Stress and Myopathy in RyR1Y522S/wt Mice [19]:

1) In case of MHS-related *RYR1* mutatons, RyR1 channels are more sensitive and open more readily to voltage, ligand, and Ca^{2+} activation, causing a Ca^{2+} leak and small, possibly local increases in resting [Ca^{2+}]; **2)** Increased cytosolic [Ca^{2+}] levels stimulates production of reactive nitrogen species generation (RNS) and **3)** subsequent increased RyR1 S-nitrosylation, which; **4)** in turn increases RyR1

temperature sensitivity. S-nitrosylation also decreases RyR1 sensitivity to Ca^{2+} inhibition further promoting SR Ca^{2+} leak; this results in **5)** additional Ca2+ increases and further enhance ROS/RNS production, leading to a vicious feed-forward cycle mechanism. Finally, chronically elevated levels of Ca^{2+} and ROS/RNS damage mitochondria and contribute to the development of myopathy. **6)** In case of heat stress – due to exercise in hot environments - Ca^{2+} release from the modified RyR receptors is greatly and persistently augmented, eventually leading to heat stroke. [19] In addition, viral infection and (medical and / or recreational drugs) may reinforce this feed-forward cycle mechanism.

Table 1: Overview of *RYR1* mutations associated with non-aesthetic rhabdomyolysis (RM) reported in the literature.

Sex	Race	Mutation(s)	EMHG causative mutation	Episodes of RM	Age at first episode	Trigger(s)	Heat stroke(s)	Fatal Exertional myalgia	CHCT / IVCT	Highest CK	Intercurrent CK	Muscle biopsy	Details of phenotype	Reference
m		**R163C**	Yes		12	E			MHS		53	Normal	Myoglobinuria; myalgia	[20]
m		**R163C**	Yes	1	13	E,H	1	yes yes	ND	9049		Normal	Heat stroke in heat	[31]
m	C	**c.957+5_957+29del (r.(spl?))**		>5	<20	E		yes	ND	8866	1130		Exertional myalgia and recurrent episodes of rhabdomyolysis	[8]
m		**G341R**	Yes		18	E		yes	MHS	ND	406	Aspecific	Myoglobinuria; myalgia	[20]
f		**R401C**		1		E		yes	MHS	5212	203	Regeneration	Exertional myalgia and cramps, and rhabdomyolysis	[26]
m		**R401C**		1		E,H		yes	MHS	899			Exertional myalgia and cramps	[26]
m	C	**R830W A3407T**		1	27	E,H	1		MHN	411.600	84	Regeneration	Heat stroke after exercise	[1]
f		**D849N**		1	20	D[1]			MHN	21.229			MDMA-induced rhabdomyolysis and malignant neuroleptic syndrome	[24]
m	AA	**A933T S1342G A1352G**		>1	21-41	E		yes	MHS	1200			Recurrent rhabdomyolysis and muscle pain	[21]
m	AA	**A933T S1342G A1352G**		>1	21-41	E		yes	MHS	1200			Recurrent rhabdomyolysis and muscle pain	[21]
m	AA	**S1342G**		>1	21-41	E		yes	MHS	6068			Recurrent rhabdomyolysis and muscle pain	[21]
m	AA	**S1342G**		1	21-41	E			MHS	10.000			Single episode of exertional rhabdomyolysis with myoglobinuria	[21]
m	AA	**S1342G**		1	30	E,D[2]	1		MHS	10.609	417		Rhabdomyolysis and compartiment syndrome; MH during operation	[25]
m	AA	**S1342G A1352G T4288_A4290dup T4294M**		1	21-41	E			MHS	100.000			Exertional rhabdomyolysis	[21]
m	AA	**S1342G G2160SS**		>1	21-41	E			MHS	4481			Recurrent rhabdomyolysis with myoglobinuria	[21]
m	C	**K1393R**		>3		E,H,D[3]		yes	ND	3.849	96		Recurrent episodes of	[8]

													rhabdomyolysis	
m	C	**K1393R R4737Q**		>2	38	E		yes	MHS	4.800	65		Recurrent episodes of rhabdomyolysis	[8]
m		**K1393R**		1	30	E,H							Heat stroke after exercise	[22]
m	C	**D2129N**		1	64	E,H		yes	ND	12.670	769	Necrosis; few cores on EM	Exertional myalgia and rhabdomyolysis	[8]
m	C	**G2132S**		>3		E,H		yes	ND	12.000	299		Exertional myalgia and recurrent episodes of rhabdomyolysis	[8]
m	C	**T2206M**	Yes	2	40	I			MHN	521.500	214	Unevenness (OS)	Recurrent episodes of infection-induced rhabdomyolysis	[1]
m	C	**I2321V V4849I**		4	15	E,H,D[4]			MHS	29.914	614		Recurrent episodes of exertional rhabdomyolysis	[35]
m	AC	**Y2426C**		2	35	E,D[5]		no	ND	44.500	150		Two episodes of rhabdomyolysis	[8]
m		**G2434R**	Yes	>1	37	E		yes	MHS		44	Normal	Recurrent episodes of fever, myoglobinuria and myalgia	[20]
f	C	**G2434R**	Yes	>6	14	E,I		yes	ND	378.900	184	Unevenness (OS)	Recurrent episodes of rhabdomyolysis	[8]
m	C	**G2434R**	Yes	1		E		no	MHS	14.765	157		Exertional rhabdomyolysis	[8]
m	AA	**R2454C**	Yes	1	30	E,H	1	yes	MHS	3.900			Exertional rhabdomyolysis	[34]
m	*C*	**A3407S**		1		E			MHS	ND	144	Mildly myopathic	MH and EIR	[23]
m		**R3983H**		1	5	D[5], I		yes	ND	3297		Multiminicore disease	MH at age 3 and rhabdomyolysis	[32]
m		**R3983C**		>1	1	E,I	>1	yes	yes	ND	100.000	Mildly myopathic	MH and recurrent rhabdomyolysis	[29]
f		**R3983C D4505H**		2	4	E,H,I	2	yes	yes	MHS			Two episodes of heat stroke	[29]
m	A	**T4288_A4290dup**		>3		E			yes	ND				[8]
m	AC	**T4288_A4290dup**		1		E,H			yes	ND	1.533	ND	Exertional rhabdomyolysis	[8]
m	A	**T4288_A4290dup T4288_A4290dup**		>10		E			yes	ND	234.417	120	Recurrent episodes of exertional rhabdomyolysis	[8]
m	C	**A4295V**		1		E			no	ND	179.600	40	Rhabdomyolysis	[8]
f		**L4320 R4322dup R4645Q**		1	2	H	1	yes		ND		Normal	Fatal heatstroke	[30]
m		**G4820**		1	6	E,H,D[6]	2	yes	yes	MHS	981	CCD (father)	Fatal heatstroke	[33]

Table 1

Sex: m = male; f = female

Race: A = African; AA = African-American; C = Caucasian; AC = Afro-Caribbean

Mutation(s): amino acid changes unless otherwhise specified

Triggers: E = exercise; H = heat; I = infection; D = drugs; [1] MDMA, haloperidol; [2] simvastatin; [3] alcohol; [4] caffeine gel; [5] olanzapine; [6] succinylcholine

CHCT: caffeine halothane contraction test / IVCT: in vitro contracture test (in patient or in one of the related *RYR1* mutation carriers; ND: not determined (mostly in cases in which previously reported mutations were detected)

Muscle biopsy: MmD = Multi-minicore Disease; CCD = Central Core Disease; OS = oxidative staining

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